XXXIII. A GENERAL METHOD OF ESTIMATING THE RELATIVE TURBIDITY OR OPACITY OF FLUID SUSPENSIONS INCLUDING BAC- TERIAL EMULSIONS.

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Methods of estimating the turbidity or opacity of fluids have been employed by chemists for some years past. T. W. Richards [1895] was the first to describe an apparatus, the "nephelometer," designed for this purpose. Later P. A. Kober [1913] found it possible to estimate turbidity with a modified Duboscq colorimeter; and the same instrument has been employed in varying modifications by Marriott [1913], Folin and Dennis [1914], W. R. Bloor [1914] and others. Dreyer and Hansen [1907] made use of the turbidity, or rather the "clarification," of fluids in an investigation of the effects of light upon enzymes etc. They carried out their experiments in dwarf test-tubes, arranged in series to contain diminishing concentrations of the active substance in a constant volume of substrate, and they found that, when dealing with a turbid substrate, the extent of the reaction could be ascertained by reading the degree of clarification of the fluid. This procedure, although used for a quite different purpose, embodies the main features of the method described in this paper.

Measurements of the turbidity or opacity of fluid suspensions have hitherto lain almost exclusively within the province of pure or applied chemistry, and no great effort has been made to employ accurate methods of this kind in the service of bacteriology. Nevertheless there are numerous bacteriological procedures which would be facilitated by an accurately quantitative method of turbidity estimation; and for one process in particular

it is absolutely essential to use a method of this kind. We refer to standardisation of agglutinable cultures. During the last twelve months the particular macroscopic method of agglutination, described by one of us [Dreyer, 1909] has come widely into use. The most essential feature of the method is the employment of sterile formalised and standardised bouillon cultures of the bacteria. The standardisation process is a double one, and concerns firstly the opacity or turbidity of the culture, and secondly its specific sensitiveness to agglutination.

The second part of the process does not concern us here [see Davison, 1916]. The measurement of the turbidity of the cultures is of great importance, because upon it depends the ease and accuracy with which their relative sensitiveness to agglutination can be estimated; for it is very difficult to compare a thin culture with a thick one, or *vice versâ*, whereas the sensitivity-estimation of cultures of equal thickness presents, as a rule, little difficulty. The turbidity-test does not necessarily provide an accurate measurement of the number of bacteria in a bouillon culture, for the total turbidity will include any initial opalescence of the bouillon in which the bacilli are grown. The difference in sensitiveness of the cultures is indicated by the sensitivity-constants of the individual cultures.

The first apparatus which we employed for turbidity-standardisation was the Duboscq colorimeter modified in a new way to serve our special purpose. Within certain limits this yielded very satisfactory results, but a serious obstacle to uniformity and certainty of the readings was encountered in the fact that our veal-bouillon cultures were not of uniform colour. The deeper the colour of the fluid, the lower were the readings for a given degree of turbidity. Various means of overcoming this difficulty suggested themselves, and of these one promised considerable success; viz. the addition of a strong deep-brown peptone solution to the paler of the two fluids to equalise the colours. But after a considerable number of these experiments had been carried out the method was discontinued and replaced by the much simpler and more accurate method described below.

General Description of Method. A graduated series of dilutions of each fluid is made in miniature test-tubes in practically the same manner as in the determination of the agglutinability. By artificial light against a dark background the turbidities of a number of tubes of one series are matched in succession with tubes of the other series. Each reading provides figures from which the relative turbidities can be calculated, and by taking the average of a number of readings an accurate ratio is obtained.

Details of Method. Apparatus. Almost any variety of dwarf test-tube or agglutination-tube may be used. It is essential to have at hand a fairly large supply of tubes, so that a selection may be made of tubes of practically equal internal diameter. The tubes must be quite clean and their surface must be free from scratches. We have been employing for the purpose two varieties of tubes which are made by Baird and Tatlock, Ltd. for the National Medical Research Committee, viz. (1) A thin-walled dwarf test-tube about 7.0 cm. long and 0.95 to 1.0 cm. in diameter. (2) A smaller "agglutination tube" 5.7–5.8 cm. in length, having an internal diameter of 5.5–6.2 mm. When the greatest possible accuracy is desired, it is best to use the larger form of tube and to measure the fluids with a 2.0 cc. pipette, accurately graduated in hundredths of a cc. But for ordinary purposes the small agglutination tubes may quite well be employed, and the dilutions made with a dropping pipette. In this latter method there is a certain error due to the difference in size of the drops of 0.85 % NaCl solution and of culture. About 17–18 drops of culture are equal to 15 drops of saline. But in dealing with small volumes of fluids, it is open to doubt whether measurements with a small graduated pipette are any more accurate than this.

Technique (when small agglutination-tubes and a dropping pipette are employed). Two sets of eleven gauged tubes are placed in a suitable stand. Into the tubes of each set the different fluids are measured as follows:

Tube	Saline	Culture
(1)	0	20
(2)	4	16
(3)	8	12
(4)	10	10
(5)	12	8
Culture diluted 1 in 2		
(6)	6	14
(7)	8	12
(8)	10	10
(9)	12	8
(10)	14	6
(11)	16	4

This series of dilutions is similar to that described for the serum-dilution in the standardisation of agglutinable cultures, only multiplied by two. It will be seen that the range of dilutions in the series is from 1/1 to 1/10, and that the progressively diminishing quantities of culture are always made up to the same volume with saline. The mixtures in the tubes are well shaken with the finger over the mouth of the tube, and the readings are then taken.

Readings. Artificial light and a black background are essential. It has

been our custom to use a frosted electric bulb with a large green shade, and some pieces of dull black boarding or paper placed on the table below the lamp and propped up behind it. A darkened room, though not absolutely necessary, adds considerably to the ease and accuracy of the readings. The lamp shade is inclined so that the bulb is just hidden from the eye, and the tubes to be compared are held up against the edge of the shade. Before reading, the outside of the tubes must be wiped with the moistened corner of a glass-cloth and then polished with the dry part of the cloth. From one series a tube is chosen at random which we call the "standard" tube for the first reading. With this the tubes of the other series are compared one after the other until one is found which matches it in turbidity. It will frequently be found that there is no tube in the second series which gives an exact match, but that there is one tube which is a little more turbid, while the next lower is a little less. In this case the standard tube is recorded as lying between those two tubes of the other series.

Moreover it is usually possible to judge whether it is nearer to one than to the other. If so, we make a mark or marks under the number of the tube to which it is nearer. Five or six readings should be made in this manner, taking a number of tubes in succession as standard. It is well to read in both directions; *i.e.* to take tubes from each series as standard. Now, since we know the dilution of the culture in every tube, it needs only a simple calculation to arrive at the relative turbidity of the two cultures. Records of typical routine estimations are given in Tables I to IV. Experiment III was performed with small tubes and a dropping pipette; I and II were made with larger tubes and a 2.0 cc. pipette. When the latter procedure is followed there is no need to make a 1 in 2 dilution of the culture for the latter part of the series. Moreover twelve tubes may be used with advantage instead of eleven.

Thus: culture (cc.) 1.0, 0.8, 0.6, 0.5, 0.4, 0.35, 0.3, 0.25, 0.2, 0.17, 0.13, 0.10. Water (cc.) 0.0, 0.2, 0.4, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.83, 0.87, 0.9. The range of dilution is the same as before; and the only difference is that the series is fuller by one tube at the right-hand end, which renders readings in this neighbourhood more accurate.

Moderate variations in the colour-intensity of the fluids do not appreciably affect the accuracy of the readings, for the progressive dilution renders such differences negligible at an early point in the series. If one of the fluids under comparison is so thick that the range of dilutions described proves insufficient to allow a comparison with the other fluid; then a further series

of tubes must be set up in precisely the same manner, but using the thicker fluid diluted (1 in 10 or 1 in 20) instead of at full strength.

In practice it is often convenient to make a rough estimate of the relative thickness of the fluids before proceeding to the complete test. This is easily done by taking a small quantity of the two fluids in two tubes of equal calibre, and diluting the thicker fluid until the two show approximately equal turbidity. From this rough test it is easy to make an approximate calculation of the relative thickness of the fluids. If one fluid appears to be four or five times thicker than the other, it is best to dilute it suitably before putting up the test-series.

TABLE I.

Estimation of relative turbidities of fluids A and B.

A is a stock "standard" culture.

B is a fresh, undiluted, formalised bouillon culture of *B. Typhosus*.

Technique: graduated 2.0 cc. pipette and large tubes.

Readings		Actual quantities of culture in the matched tubes		Ratio	Deviation from mean in %
Series A Tube	Series B Tube	A	B		
2 matches	7	0.80 cc.	=0.30 cc.	2.67	0.37
3- 4	9	0.53*	=0.20	2.65	1.12
4 -5	10	0.46	=0.17	2.71	1.12
2- 3	8	0.67	=0.25	2.68	0.00
5 -7	11	0.35	=0.13	2.69	0.37
		Mean 2.68		Mean error 0.60 %	

* This figure is obtained by interpolation. The reading 3-~~4~~ signifies "between tubes 3 and 4, but rather nearer 4." Now tube 3 contains 0.60 cc. and tube 4 contains 0.50 cc.—so we may take the figure to be a little less than 0.55, viz. 0.53.

The average-figure given at the foot of the third column is an expression of the fact that 1.0 cc. of *B* gives the same turbidity-reading as 2.68 cc. of *A*, when both are diluted to the same volume. If we now wish to dilute *B* to the same turbidity as *A*, all we have to do is to add to each 1.0 cc. of *B* 1.68 cc. of diluting fluid.

The readings vary, as a rule, about 3 % to 5 % on either side of the mean, and it is uncommon to find a reading with an error so great as 10 %. Such a close correspondence of the individual readings, each of which is in itself an experiment, is sufficient evidence of the trustworthiness of the method.

TABLE II.

*Estimation of relative turbidities of fluids A and B.**A* is a stock standard culture.*B* is a fresh, undiluted, formalised broth culture.

Technique: graduated 2.0 cc. pipette and large tubes.

Readings		Actual quantities of culture in the matched tubes		Ratio	Deviation from mean in %
Series A Tube	Series B Tube	A	B		
5 matches	9	0.40 cc.	= 0.20 cc.	2.00	0.50
2 "	5	0.80	= 0.40	2.00	0.50
6 "	10	0.35	= 0.17	2.06	2.49
3 "	7	0.60	= 0.30	2.00	0.50
7 "	10-11	0.30	= 0.15	2.00	0.50
				Mean 2.01	Mean error 0.90 %

Calculation for diluting *B* to the same turbidity as *A*:1.0 cc. of *B* = 2.01 cc. of *A*.Therefore, to each 1.0 cc. of *B* add 1.01 cc. of diluting fluid.

TABLE III.

Method: small Dreyer's agglutination tubes and dropping pipette.

A is a stock standard culture.*B* is a fresh, undiluted formalised bouillon culture.

Readings		Actual quantities of culture in the matched tubes		Ratio	Deviation from mean in %
Series A Tube	Series B Tube	A	B		
4 matches	9	0.500 cc.	= 0.200 cc.	2.500	0.16
3 "	8-9	0.600	= 0.245	2.450	2.16
2 "	6-7	0.800	= 0.315	2.540	1.44
1 "	5-6	1.000	= 0.395	2.530	1.04
6 "	10-11	0.350	= 0.140	2.500	0.16
				Mean 2.504	Mean error 0.99 %

Although we have paid special attention to the use of this method in the preparation of standard agglutinable cultures, the method itself is not limited to any such special use. As an instance of its application in quantitative chemistry we give in Table IV an experiment in the estimation of minute quantities of silver chloride in suspension or colloidal solution.

The close correspondence between the values obtained by our turbidity-method and the values calculated from the known strengths of the solutions demonstrates that this simple technique is at least as accurate as the more elaborate methods of turbidity-estimation hitherto employed in the quantitative measurement of dilute inorganic solutions.

Description of Experiment shown in Table IV. The estimation of the relative strength of two dilute AgNO_3 solutions—by quantitative conversion into AgCl and turbidity-measurement. The AgNO_3 solutions were kindly prepared for us by Mr N. V. Sidgwick, and were labelled A = between $N/100$ and $N/1000$, and B = between $N/1000$ and $N/10,000$. Their true concentrations, which were unknown to us until after the experiment was completed, were:

$$A = 0.001254 \text{ N AgNO}_3,$$

$$B = 0.0004013 \text{ N AgNO}_3.$$

After a rough preliminary experiment, solution A was diluted one in four, to bring it down to a strength sufficiently near that of solution B . A thus diluted is referred to as A (0.25).

To 10 cc. of solution A (0.25) 10 cc. of 0.85 % NaCl solution was added.

To 10 cc. of solution B 10 cc. of 0.85 % NaCl solution was added.

The mixtures were kept for four hours in a cool and dark place, and then the resulting suspensions (or colloidal solutions) of AgCl were subjected to a turbidity-estimation in the usual manner. Both solutions showed a bluish opalescence without any visible trace of flocculation. Large tubes and a graduated 2.0 cc. pipette were employed for the measurements.

TABLE IV.

Turbidity-estimation of two silver chloride suspensions (or colloidal solutions).

A and B are two AgNO_3 solutions, converted quantitatively into AgCl (see text).

Readings A (0.25) B Tube	Actual quantities of AgCl suspensions A (0.25) B		Ratio observed $\frac{0.25A}{B}$	Deviation from mean in %	Ratio calculated $\frac{0.25A}{B}$
1 = 2 -3	100	76	1.316	+2.41	$A = 0.001254 \text{ N AgNO}_3$ $B = 0.0004013 \text{ N AgNO}_3$ $\frac{0.25A}{B} = \frac{0.0003135}{0.0004013} = 1.280$
3 = 4 -5	60	47	1.277	-0.62	
5 = 6 -7	40	33	1.212	-5.68	
7 = 8	30	25	1.200	-6.61	
7 = 8 -9	27	20	1.350	+5.06	
5 -6=7	38	30	1.267	-1.40	
3- 4 =5	52	40	1.300	+1.17	
2- 3 =4	68	50	1.360	+5.84	
Mean 1.285			Mean error 3.60		1.280

Percentage divergence of observed from calculated ratio -0.39.

APPENDIX.

Gauging of the Tubes. For the selection of tubes of almost equal bore, a gauge is necessary. The following type has been found very convenient (Fig. 1). From a thin strip of brass a piece about an inch long is cut in the shape of a longitudinal section of a long truncated cone. The narrower end has the width of the minimum internal diameter allowable for the selected tubes (see below) and the wider end, to which a brass rod is soldered as a handle, has the width of the maximum internal diameter permissible. The gauging of the tubes consists in choosing those tubes whose mouths permit the entry of the narrower end of the gauge but refuse to admit the broader end. (A temporary but perfectly serviceable gauge of this kind can quickly

Fig. 1. Composite gauge for agglutination-tubes and dropping pipettes.

At one end of the instrument is seen the maximum (*A*) and minimum (*B*) gauge for the internal diameter of the agglutination-tubes. This is the only part that is described in the text. The holes in the plate at the other end are for the gauging of the external diameters of the tubes and of the capillary end of the dropping-pipettes. This part of the instrument is not essential for the method we have described.

and easily be cut from a small strip of thin wood to any designed measurement.) For the larger tubes the maximum and minimum measurements of the gauge may conveniently be made 9.3 mm. and 9.0 mm. respectively; and for the smaller tubes 5.7 mm. and 6.0 mm. respectively.

Although this type of gauge measures only the mouth of the tube, and gives no information about its lower end, we have not found that this introduces any appreciable error, since the tubes are not long enough to show any considerable variation in width from end to end.

It is hardly necessary to say that the more uniform the bore of the selected tubes is, the greater will be the accuracy of the turbidity-readings.

SUMMARY.

A description has been given of a simple and accurate method of measuring the relative turbidity or opacity of two fluids; and its special application to the standardisation of agglutinable cultures has been described in detail.

The method is of general application in all chemical and biological investigations which require the measurement of turbidity.

The technique is extremely simple and no special or expensive apparatus is needed.

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